TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED / ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371	ATTORNEY'S DOCKET NUMBER P65952US0
	US APPLICATION NO. (if known, see 37 CFR 1.5) 09/623495

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB99/00820	17 March 1999	18 March 1998

TITLE OF INVENTION

AMORPHOUS GLASSES FOR STABILIZING SENSITIVE PRODUCTS

APPLICANT(S) FOR DO/EO/US

Bruce Joseph ROSER; Areadio Garcia DE CASTRO

Applicant herein submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for Internatl. Preliminary Examination was made by the 19th month from earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the Internatl. Preliminary Examination report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

    International Search Report
    PCT/IB/306 Form
    International Preliminary Examination Report
    Letter to EPO

428 Rec'd PCT/PTO 18 SEP 2000

US APPLICATION NO (If known, see 37 CFR 1.5) 09/623495	INTERNATIONAL APPLICATION NO PCT/GB99/00820	ATTORNEY'S DOCKET NUMBER P65952US0

	CALCULATIONS	PTO USE ONLY
17. ☒ The following fees are submitted:		
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Internatl. prelim. examination fee paid to USPTO (37 CFR 1.492 (a) (1)) . . $670.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (2)) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) . . $760.00		
Neither international preliminary examination fee (37 CFR 1.492 (a) (3)) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$970.00**		
International preliminary examination fee paid to USPTO (37 CFR 1.492 (a) (4)) and all claims satisfied provisions of PCT Article 33(2)-(4) $96.00		
Search Report prepared by the EPO or JPO (37 CFR 1.492 (a) (5)) **$840.00**	$ 840.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =		
Surcharge of $130.00 for furnishing the **oath or declaration** later than ☐ **20** ☒ **30** months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	**Number Filed**	**Number Extra**	Rate		
Total Claims	**15** - 20 =	-0-	x $18.00	$	
Independent Claims	**1** - 3 =	-0-	x $78.00	$	
Multiple Dependent Claim(s) (if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 970.00	
Reduction by 1/2 for filing by **small entity**, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 970.00	
Processing fee of $130 for furnishing the **English translation** later than ☐ **20** ☐ **30** months from the earliest claimed priority date (37 CFR 1.492(f))				$	
TOTAL NATIONAL FEE =				$ 970.00	
Fee of $40.00 for recording the enclosed **assignment** (37 CFR 1.21(h)). Assignment must be accompanied by appropriate cover sheet (37 CFR 3.28, 3.31).				$	
TOTAL FEES ENCLOSED =				$ 970.00	
				Amt. to be refunded:	$
				Amt. charged:	$

a. ☒ A check in the amount of $ 970.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. **06-1358** in the amount of $ --- to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge my account any additional fees set forth in §1.492 during the pendency of this application, or credit any overpayment to Deposit Account No. **06-1358**. A duplicate copy of this sheet is enclosed.

SEND ALL CORRESPONDENCE TO:
Jacobson, Price, Holman & Stern, PLLC
400 7th Street, N.W., Suite 600
Washington, DC 20004
202-638-6666
CUSTOMER NUMBER: 00136

By Jonathan L. Scherer
Jonathan L. Scherer
Reg. No. 29,851

JPH&S 3/95

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Roser, et al.
International Serial No.:	PCT/GB99/00820
International Filing Date:	17 March 1999
U.S. Filing Date:	18 September 2000
For:	AMORPHOUS GLASSES FOR STABILISING SENSITIVE PRODUCTS

PRELIMINARY AMENDMENT TO LESSEN FEES

Assistant Commissioner of Patents
Washington, D.C. 20231

Sir:

Prior to initial examination, please amend the above-identified application as follows:

IN THE CLAIMS

Claim 4, line 1, delete "any of claim 1 to 3",
insert --claim 1--;
Claim 6, line 1, delete "any of claim 1 to 5",
insert --claim 1--;
Claim 10, line 1, delete "any preceding claim",
insert --claim 1--;
Claim 11, line 1, delete "any preceding claim",
insert --claim 1--;
Claim 13, line 1, delete "any preceding claim",
insert --claim 1--;
Claim 14, line 1, delete "any preceding claim",
insert --claim 1--;
Claim 15, line 1, delete "or 7".

REMARKS

The foregoing Preliminary Amendment is requested in order to delete the multiple dependent claims and avoid paying the multiple dependent claims fee.

Early action on the merits is respectfully requested.

Respectfully submitted,
JACOBSON, PRICE, HOLMAN & STERN, PLLC

By Jonathan L. Scherer
Jonathan L. Scherer
Reg. No. 29,851

400 Seventh Street, N.W.
Washington, D.C. 20004-2201
(202) 638-6666
Date: September 18, 2000
Atty. Docket: P65952US0
JCH/JLS/peg

AMORPHOUS GLASSES FOR STABILISING SENSITIVE PRODUCTS

Drying of biological molecules such as foodstuffs, vaccines and drugs is an ancient way of preserving them. However, the price that often has to be paid for increased stability is product damage and a decrease in quality. More recently it has been learned that certain additives can prevent this damage. Levine and Slade were amongst the first to realise that the best additives were substances that tended to solidify from solution as an amorphous glass rather than by forming crystals (Levine H. and Slade L., Principles of cryostabilisation technology from structure/property relationships of carbohydrate/water systems: A review. *Cryoletters* 9 21-63 (1988)). Foremost amongst the stabilisers they recommended were certain sugars, notably sucrose. Although not formally proved, the stability of these dried formulations is assumed to be a function of the transformation of the sugar solution into a glass as it dries. As a consequence of the progressive increase in viscosity of the sugar syrup, it is thought that the active molecules are concentrated by a smooth progression from their mobile native state in liquid solution to an immobilised solid solution or glass. In the glass, molecular motion and hence diffusion-based molecular interactions are negligible. The chemistry responsible for degradation is arrested and the product remains stable as long as it remains dry and glassy (Franks F., Freeze drying: From empiricism to predictability. *Cryoletters* 11 93-110 (1990)).

The simple view that glass formation is the explanation for stability is incomplete. This explanation only holds for glasses that are inherently chemically unreactive and stable. It is incontrovertible that certain substances that form physically excellent glasses are nevertheless very poor stabilisers. Many of these are either chemically reactive, such as reducing sugars or are unstable, like sucrose or sorbitol and break down to reactive intermediates which degrade the product during storage in the dry state (Newman Y.M., Ring S.G. and Colaco C. The role of trehalose and other carbohydrates in biopreservation. *Biotechnol. & Genet. Eng. Rev.* 11 263-294 (1993)). A common reactivity of sugar glasses is the reducing action of the carbonyl group and the degradation products formed are often the familiar carbonyl-amine compounds of the Maillard reaction (Ellis G.P. The Maillard reaction. *Adv. Carbohyd. Chem.* 14 63-134 (1959)). Because the Maillard reaction is temperature dependent, it is only slowly progressive at low temperatures. Many glass-forming materials give excellent preservation of activity during the drying process

itself but the product subsequently undergoes progressive deterioration unless stored under refrigeration.

The error of ignoring the subtle sugar chemistry that can proceed in dried preparations is widespread in the literature and has led to the advocacy of simple tests of the glass transition temperature of pure sugar solutions as a means of selecting good stabilisers. This approach has actually led to the recommendation of quite useless substances in the past (Franks F. Freeze drying: From empiricism to predictability *Cryoletters* 11 93-110 (1990)). In fact, the efficacy of a formulation is the result of multiple physical and chemical interactions between all the components in the formulation, including the active, on drying. All these interactions may not be predicted by current theories.

The superiority of the disaccharide trehalose as a stabiliser was first indicated by its prevalence in certain rare living creatures, which regularly dried out and could come back to life on rehydration. (Crowe J.H., Crowe L.M. and Chapman D. Preservation of membranes in anhydrobiotic organisms: The role of trehalose. *Science* **223** 701 (1984)). In laboratory studies, trehalose incorporated into the buffer solutions from which active biomolecules were dried, resulted in a product with quite remarkable resistance to denaturation by heat (Colaco C.A.L.S., Sen S., Thangavelu M., Pinder S. and Roser B. Extraordinary stability of enzymes dried in trehalose: Simplified molecular biology. *Biotechnol.* **10**. 1007-1011 (1992)). Because it is subsequently degraded in the body by the specific enzyme, trehalase, to two molecules of glucose, trehalose possesses many of the properties of the ideal industrial stabiliser for foods and medical products. A large scientific and patent literature has now developed on trehalose stabilisation of foods, vaccines, diagnostics and drugs. The disadvantages of trehalose are that it is as yet not approved by the regulatory authorities, it is expensive and it contains contaminating reducing sugars, especially glucose, in all but the most rigorously purified material.

One of the alternative non-reducing and chemically stable sugar derivatives that might be expected to stabilise effectively is mannitol. Because of its remarkable resistance to water sorption at high atmospheric humidity (Wade A. and Weller P.J. Handbook of Pharmaceutical Excipients second edition p296 (1994)), it is widely used in tablet and powder formulations as a bulking and anti-caking agent. In combination with other excipients such as glycine, it is also widely used in freeze dried parenteral preparations but

is added as a carrier to produce a stiff, homogeneous cake that improves the appearance of the lyophilised plug in a vial (Wade & Weller The Excipients Handbook 1994).

In published surveys of the stabilising ability of a wide range of sugars and sugar derivatives (Colaco C.A.L.S., Smith C.J.S., Sen S., Roser D.H., Newman Y., Ring S. and Roser B.J. Chemistry of protein stabilisation by trehalose in *Formulation and delivery of proteins and peptides* Cleland and Langer eds American Chemical Society Washington 222-240 (1994)), it was shown that mannitol and sorbitol were very poor stabilisers. Indeed in several patent applications it has been claimed that mannitol and certain other monosaccharide alcohols cannot stabilise at all. PCT No WO 91/18091 "Stabilisation of biological macro-molecular substances and other organic compounds", Roser B.J. and Colaco C claimed that only non-reducing glycosides of a polyhydroxy sugar alcohol or other straight chain polyalcohol or raffinose, stachyose or melezitose were effective in achieving stability, especially on storage. This patent states "Thus the monosaccharide sugar alcohols galactitol, mannitol and erythritol are not satisfactory protective agents". US patent Number 5,621,094 "Method of Preserving Agarose Gel Structure During Dehydration by Adding a Non-reducing Glycoside of a Straight Chain Sugar Alcohol" by Roser B. and Colaco C states that "glucose mannitol and sorbitol failed after one week" while "lactitol and trehalose were perfect after >12 weeks". It further defined effective formulations as "wherein the non-reducing glycoside of a straight chain sugar alcohol does not form crystals during dehydration". PCT No WO 96/05809 "Improved method for stabilisation of biological substances during drying and subsequent storage and compositions thereof", by Colaco C., Roser B.J. and Sen S. claims methods wherein even reducing sugars are used to stabilise products. This is achieved by preventing the sugars from attacking the product by using Maillard reaction inhibitors. This application states that mannitol has no stabilising effect whatsoever.

We have now found this to be incorrect. In contradiction to the statements in these documents we have found that certain monosaccharide sugar alcohols such as mannitol and inositol can be excellent stabilisers when correctly formulated and in fact have significant advantages over trehalose for some applications. In view of mannitol's acceptance by regulatory authorities and widespread use in the healthcare industry in both parenteral and oral formulations, it has considerable advantages as a new stabilising excipient. Its low cost and chemical inertness, together with its exceptional stability and

its high purity and safety, would make it the stabiliser of choice for pharmaceuticals. We have found that certain substances must be present in a formulation to convert mannitol into an excellent stabiliser. The effect of these substances is dose dependent and below a threshold concentration they do not work. The substances useful in accordance with this invention promote the drying of mannitol solutions as glasses rather than crystals. One of the most potent materials is the borate ion either as boric acid, or tetraborate salts of sodium or potassium. This probably forms a network complex with mannitol or even a covalent compound, sodium mannitoborate. Subsequent to the filing of this application containing the disclosure of the surprising efficacy of small amounts of borate in inducing glass formation in drying mannitol solutions, similar beneficial effects with borate and trehalose were reported by Miller et al. Notably, the molar ratio of borate to trehalose used by these authors was considerably higher than we found to be necessary. (Miller DP, Anderson RE and de Pablo JJ. Stabilisation of lactate dehydrogenase following freeze-thawing and vacuum-drying in the presence of trehalose and borate. *Pharmaceutical Research* 15 1215-1221 (1998)). Other effective materials such as calcium lactate, and proteins such as serum albumin or gelatin, or polyamine materials such as polyvinyl pyrollidone, or polyvinyl alcohol, intrinsically form glasses themselves when dried from solution. Yet other effective chemicals such as acetate salts, will form glasses but only when quenched from the melt and only when the melt contains several metal cations rather than a single cation, such as sodium and calcium. An additional property of the materials identified to date is that the beneficial actions of these materials are additive so that they can be mixed together in successful formulations which contain sub-threshold doses of each additive alone. Other substances which are either themselves glass-formers (under certain conditions) or are glass-formation-facilitators such as the phosphate salts of sodium and potassium and sodium silicate are capable of being utilised to make stabilising glasses according to this invention.

The quality of the glasses made by this process is high. The glass transition temperature (Tg) of 1:1 w/w mannitol/calcium lactate glass is around 68°C (Figure 1). This compares with a Tg of around 90°C for a trehalose/sodium sulphate glass dried under the same conditions (Figure 2). Both types of glass have Tg's well above any possible ambient storage temperature and, because the glasses are chemically inert and non-reactive, the entrapped products are stable at room temperatures and require no refrigeration of any kind.

It is a particular advantage of this invention that sugars which have previously been known as stabilisers in methods of freeze drying may be used successfully in drying compounds subject to deactivation on drying whilst utilising drying temperatures above freezing point (e.g. room temperature or above). Thus US Patent 5,589,167 "Excipient stabilisation of polypeptides treated with organic solvents" by Cleland and Jones reports that mannitol and trehalose are both excellent stabilisers for the recombinant peptides human Growth Hormone and human Interferon gamma on freeze-drying and on exposure to organic solvents. These authors found that the ratio of active to excipient was critical in achieving the optimum stabilisation. Without wishing to be bound by theory, it is likely that the good results reported in this patent are not so much due to the substitution of freeze-drying for ambient temperature drying but the use of very high ratios of active to excipient in the formulations. Under these circumstances, where the mass ratio of peptide to mannitol or trehalose was 1 to 1 or higher, the peptide itself was acting as an enhancer of glass transformation of the drying sugar solutions in a manner analogous to the action of albumin or gelatin shown below. A serious disadvantage of this approach is that highly potent drugs and vaccines need tiny amounts of stabilising sugars to give a good stabilising glass. Single dose ampoules for example appear to be completely empty; the thin film of dried product being invisible in the container. This is confusing to the end user and can be wasteful, if the container is discarded in error, or even dangerous, if the time to complete dissolution of the product is not obvious after reconstitution. This greatly reduces the flexibility of formulation and presentation of the product.

What is required is a robust formulation that inherently forms a good glass even in the absence of product but which can accommodate a wide range of product concentrations without loss of glass forming capacity and stabilising efficacy. A number of sugar alcohols previously rejected as stabilising agents in the prior art listed above such as mannitol, xylitol, inositol, arabinitol and galactitol stabilise very effectively when correctly formulated so as to promote the formation of a glassy matrix, rather than crystals, on drying. A simple method for inhibiting crystallisation is to mix two or more sugars or sugar derivatives together in the same formulation. When correctly chosen, these mutually inhibit crystallisation and the mixture dries as an amorphous glass. In some cases these glasses are more robust on storage and give greater stability to an included product than trehalose itself.

Brief Description of the Figures

Figure 1 shows differential scanning calorimetry of a 50 / 50 w/w mannitol / calcium lactate glass showing a clear glass transition at a temperature of 68 °C;

Figure 2 shows differential scanning calorimetry of a 50 / 50 w/w trehalose / calcium lactate glass showing a clear glass transition at a temperature of 90 °C;

Figure 3 shows the percentage recovery of alkaline phosphatase activity after vacuum-drying in either trehalose or formula 7 containing mannitol, inositol, galactitol and degraded gelatin (Byco C) followed by storage at 37°C or 50°C for up to 6 weeks. There is no loss with formula 7 but serious losses with trehalose;

Figure 4 shows the percentage recovery of alkaline phosphatase activity after freeze-drying in either trehalose or formula 7 containing mannitol, inositol, galactitol and degraded gelatin (Byco C) followed by storage at 37°C or 50°C for up to 7 weeks. There is little loss with either stabiliser;

Figure 5 shows the percentage recovery of Erythropoietin (EPO) after vacuum-drying in either trehalose or formula 8 containing mannitol, inositol, galactitol and calcium lactate followed by storage at 37°C or 50°C for up to 6 weeks. While there is serious losses with trehalose, no loss occurs with formula 8; and

Figure 6 shows the percentage recovery of EPO after freeze-drying in either trehalose or formula 7 containing mannitol, Inositol, galactitol and degraded gelatin (Byco C) or formula 8 in which calcium lactate was substituted for the gelatin. After storage at 37°C or 50°C for 7 weeks, there is no loss with any of the stabilising formulations.

Figure 7 shows the percentage recovery of alkaline phosphatase activity after spray drying formula 9 to which had been added insoluble calcium phosphate powder to increase the density of the glass microspheres. After storage at either 37°C or 55°C for up to 90 days there was no significant loss of activity.

Figure 8 shows the percentage recovery alkaline phosphatase activity after spray drying formula 11 to which had been added insoluble barium sulphate powder to increase the density of the glass microspheres. After storage at either 37°C or 55°C for up to 90 days there was again no significant loss of activity.

Examples

Example 1

A solution of mannitol in water 20% w/v was pipetted in 100 µl volumes on to the surface of clean glass microscope slides which were laid flat on a hotplate at 70°C for drying. Within about 5 min. the solution had dried into a mass of crystals. A 20% solution of trehalose or palatinit, dried under the same conditions formed a hard and transparent perfect glass film. Only the latter sugars stabilise actives successfully as described in the patents and publications referenced above. This is considered to be a function of their ability to form amorphous glass on drying.

The addition of a network forming additive such as sodium or potassium tetraborate to the mannitol solution in amounts of less than 10% of the weight of mannitol, completely inhibited crystallisation on drying and resulted in the formation of glasses as perfect as those made with trehalose or palatinit. This demonstrated that mannitol can form glasses under appropriate conditions and a search was then made for less toxic additives to achieve the same effect.

Example 2

Equal weights of trehalose or palatinit were mixed with the mannitol in solution and dried as above. In both cases this yielded perfect glasses showing that these two glass-forming sugars could inhibit the crystallisation of mannitol. Even a sugar which was not itself a glass former, such as galactitol, inhibited the crystallisation of a mannitol / inositol mixture which itself crystallised readily. Similar results were found when equal weights of other glass forming substances such as calcium lactate, albumin, polyvinyl pyrollidone or degraded gelatin (Byco C) were added to mannitol in solution. To establish the longer term stability of these glasses they were held at 70°C overnight and then at room temperature and ambient humidity for several weeks and inspected frequently. All the above glasses were stable under both sets of conditions. When other monosaccharide alcohols such as galactitol, xylitol, arabinitol, adonitol, or inositol were substituted for

mannitol, similar results were obtained but the resulting glasses were very soft when alcohols of the pentose sugars were used.

Example 3

More complex mixtures of the monosaccharide alcohols could also be blended together with glass forming substances to yield excellent glasses which showed good physical stability in the glass phase at 70°C and at ambient conditions for many weeks as described in Example 2. Some good formulations are:-

1. mannitol 33.3%, inositol 33.3% and PVP 33.3%
2. mannitol 31.6%, inositol 31.6% xylitol 5% and calcium lactate 31.6%
3. mannitol 33.3%, inositol 33.3% and calcium lactate 33.3%
4. mannitol 33.3%, inositol 33.3% and Byco C 33.3%
5. mannitol 23.3%, inositol 23.3% calcium lactate 30% and PVP 23.3%
6. mannitol 33.3%, arabinitol 33.3% and calcium lactate 33.3%
7. mannitol 30%, inositol 15% galactitol 15% and Byco C 40%
8. mannitol 30%, inositol 15% galactitol 15% and calcium lactate 40%
9. mannitol 33%, Byco C 33% and calcium lactate 33%
10. mannitol 50%, and Kollidon 30 (polyvinylpyrollidone (PVP)) 50%
11. mannitol 33%, Kollidon 30 (polyvinylpyrollidone (PVP)) 33% and calcium lactate 33%
12. mannitol 50%, and Dextran 50%
13. mannitol 33%, Dextran 33% and calcium lactate 33%

In short, simple trial and error experimentation will establish a successful formulation from mixtures of monosaccharide alcohols and a glass forming substance. By this method the final concentration of any single ingredient can be kept low. In this way a substantial total solids content can be achieved, even including sugar alcohols, which are individually not very soluble. The high solids content shortens drying times and increases the protection of the active during drying.

In addition to heat-assisted air-drying as above, formulations of this kind have been successfully vacuum dried, spray dried and freeze-dried.

Example 4

Stability of alkaline phosphatase enzyme.

Affinity purified alkaline phosphatase from bovine intestinal mucosa (Sigma Chemical Co cat No. p-8647) was vacuum-dried or freeze-dried in 100 µl volumes in formulation Number 7 above or in trehalose, and stored at 37°C or 50°C for 5 weeks. Samples were tested for activity at intervals using the Sigma assay with p-nitrophenyl phosphate as substrate. Vacuum drying was done at a shelf temperature of 40°C and a vacuum of 30-100 millitorr for 4 hr. The temperature was then ramped gradually to 60°C over 1 hr and the vials were stoppered and removed from the vacuum chamber for high temperature storage trials. Freeze-drying was done in a Labconco dryer at an initial shelf temperature of –40°C for 3 hr at a vacuum of 30-100 millitorr. The shelf temperature was then ramped to 0°C at 5°C / min and held for 1 hr. The shelf temperature was then raised to 40°C at 5°C / min and secondary drying was continued for a further 3 hr when the vials were stoppered under vacuum and removed for storage trials.

While there was some variability in the assays of enzyme activity, obvious trends were observed. Samples dried by either method without stabilisers lost all activity within a day or two of storage (not shown). Samples dried in either mannitol alone or a modified formula 7 lacking the glass forming facilitator lost between 75 and 80% of activity within 3 days at 37°C.

There was also a progressive loss of enzyme activity seen with the samples vacuum-dried in trehalose, which was not seen in the samples dried in formula 7 (Fig 3). This was particularly marked where the samples had been stored at the higher temperature (50°C). No such loss in the trehalose-dried samples was seen in the freeze-drying experiments where trehalose appeared to be slightly superior to the monosaccharide alcohols (Fig 4). This result might possibly indicate that a higher residual moisture content may have been responsible for the serious losses with trehalose in the vacuum dried samples. Whatever the explanation, it is clear that formula 7 gives results which are equivalent to, or superior to, the results obtained with trehalose.

Example 5

Stability of recombinant human Erythropoietin (EPO)

EPO was vacuum dried or freeze-dried as above in the same solutions and also in a variant of formulation 7 in which calcium lactate was substituted for Byco C (Formula 8), and then subjected to the same stability tests before being assayed by a standard 2-site sandwich Enzyme Immunoassay.

The results again showed a serious, progressive deterioration in the vacuum dried samples dried in trehalose, more dramatic at 50°C storage temperature, which was not seen with the samples dried in formula 8 (fig 5). The deterioration in trehalose was not seen in the freeze-dried samples stored at 37°C (not shown) or 50°C (Fig 6). Irrespective of whether formula 7 or 8 was used, all samples showed essentially complete recovery of activity.

Example 6

The fluorescent protein R-Phycoerythrin was air-dried in trehalose, formula 3 or formula 4 on a hotplate as described in Example 1. The intensity of fluorescence was gauged visually when illuminated with a UV lamp. In the controls dried in trehalose fluorescence was retained. The material dried in formula 3 was masked by an intense silver autofluorescence from the Byco C while the material dried in formula 4 fluoresced with the characteristic orange colour with apparently undiminished intensity.

Claims

1. A method of drying, without damage, a compound which is subject to deactivation on drying, or a mixture of such compounds, comprising subjecting an aqueous system containing the compound or mixture to drying in the presence of one or more monosaccharide sugar alcohols and at least one additive which is a glass-former or a glass-formation-facilitator, whereby the compound solidifies from solution as an amorphous glass rather than by forming crystals.

2. A method according to claim 1 in which the aqueous system contains from 0.05 to 90% by weight of sugar alcohol.

3. A method according to claim 1 in which the ratio of sugar alcohol plus additive to compound is at least 0.25:1 preferably 0.5:1 by weight.

4. A method according to any of claim 1 to 3 in which the compound is a protein, polysaccharide or nucleic acid.

5. A method according to claim 4 in which the compound or mixture comprises an enzyme, serum, serum complement, an antibody or antigen (either free or coupled to a support), a nucleic acid, a fluorescent protein, or a vaccine component.

6. A method according to any of claim 1 to 5 in which the system is dried under conditions selected from one or more of the group consisting of ambient temperature or above, chill drying, freeze drying, spray drying, vacuum drying and drying at atmospheric pressure.

7. A dried product which is an amorphous glass containing monosaccharide sugar alcohol and at least one additive which is a glass-former or a glass-formation-facilitator and a compound which is subject to deactivation on drying, or a mixture of such compounds, in a weight ratio of sugar alcohol plus additive to compound of at least 0.25:1 preferably 0.5:1, the product having been dried.

8. A dried product according to claim 7 in which the compound is a protein, polysaccharide or nucleic acid.

9. A dried product according to claim 8 containing an enzyme, serum complement, an antibody or antigen (either free or coupled to a support), a nucleic acid, a fluorescent protein, or a vaccine component.

10. A method or product according to any preceding claim wherein the sugar alcohol is selected from the group consisting of mannitol, galactitol, xylitol, arabinitol and inositol.

11. A method or product according to any preceding claim wherein there is one or a mixture of additives selected from the group consisting of peptide, protein, borate ion, calcium lactate, phosphate, silicate and acetate salts

12. A method or product according to claim 11 wherein at least one additive is selected from the group consisting of boric acid, tetraborate salt of sodium or potassium and sodium mannitoborate.

13. A method or product according to any preceding claim wherein the amorphous glass is formed from a mixture of 2 or more monosaccharide sugar alcohols.

14. A method or product according to any preceding claim wherein there is an additive which is a protein or denatured protein.

15. A method or product according to claim 1 or 7 wherein the amorphous glass is formed from a formulation having essentially a composition selected from:

1. mannitol 33.3%, inositol 33.3% and PVP 33.3%
2. mannitol 31.6%, inositol 31.6% xylitol 5% and calcium lactate 31.6%
3. mannitol 33.3%, inositol 33.3% and calcium lactate 33.3%
4. mannitol 33.3%, inositol 33.3% and Byco C 33.3%
5. mannitol 23.3%, inositol 23.3% calcium lactate 30% and PVP 23.3%
6. mannitol 33.3%, arabinitol 33.3% and calcium lactate 33.3%
7. mannitol 30%, inositol 15% galactitol 15% and Byco C 40%

8. mannitol 30%, inositol 15% galactitol 15% and calcium lactate 40%
9. mannitol 33%, Byco C 33% and calcium lactate 33%
10. mannitol 50%, and Kollidon 30 (polyvinylpyrollidone (PVP)) 50%
11. mannitol 33%, Kollidon 30 (polyvinylpyrollidone (PVP)) 33% and calcium lactate 33%
12. mannitol 50%, and Dextran 50%
13. mannitol 33%, Dextran 33% and calcium lactate 33%

ABSTRACT

Disclosed is a method of drying, without damage, a compound which is subject to deactivation on drying, or a mixture of such compounds, comprising subjecting an aqueous system containing the compound or mixture to drying in the presence of one or more monosaccharide sugar alcohols and at least one additive which is a glass-former or a glass-formation-facilitator, whereby the compound solidifies from solution as an amorphous glass rather than by forming crystals.

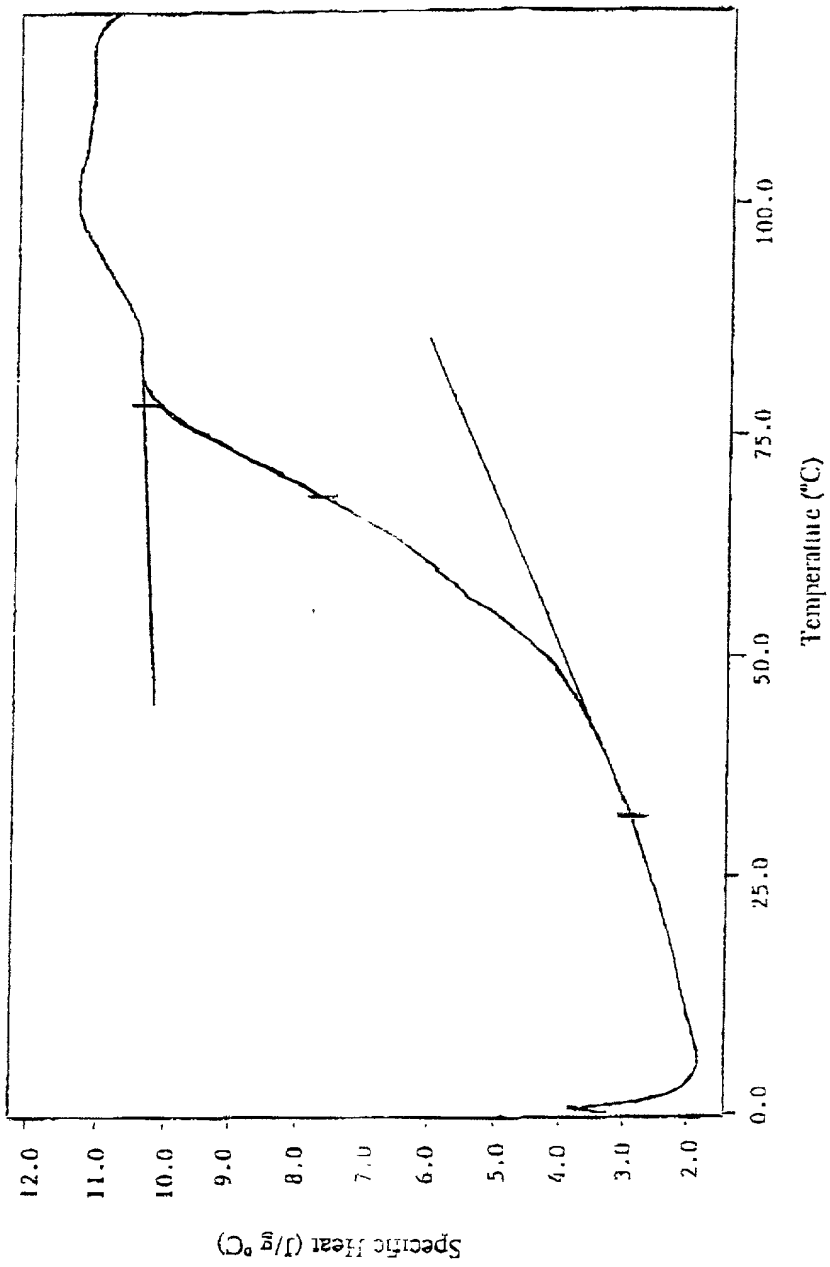


Figure 1 MANNITOL / CALCIUM LACTATE 1 : 1

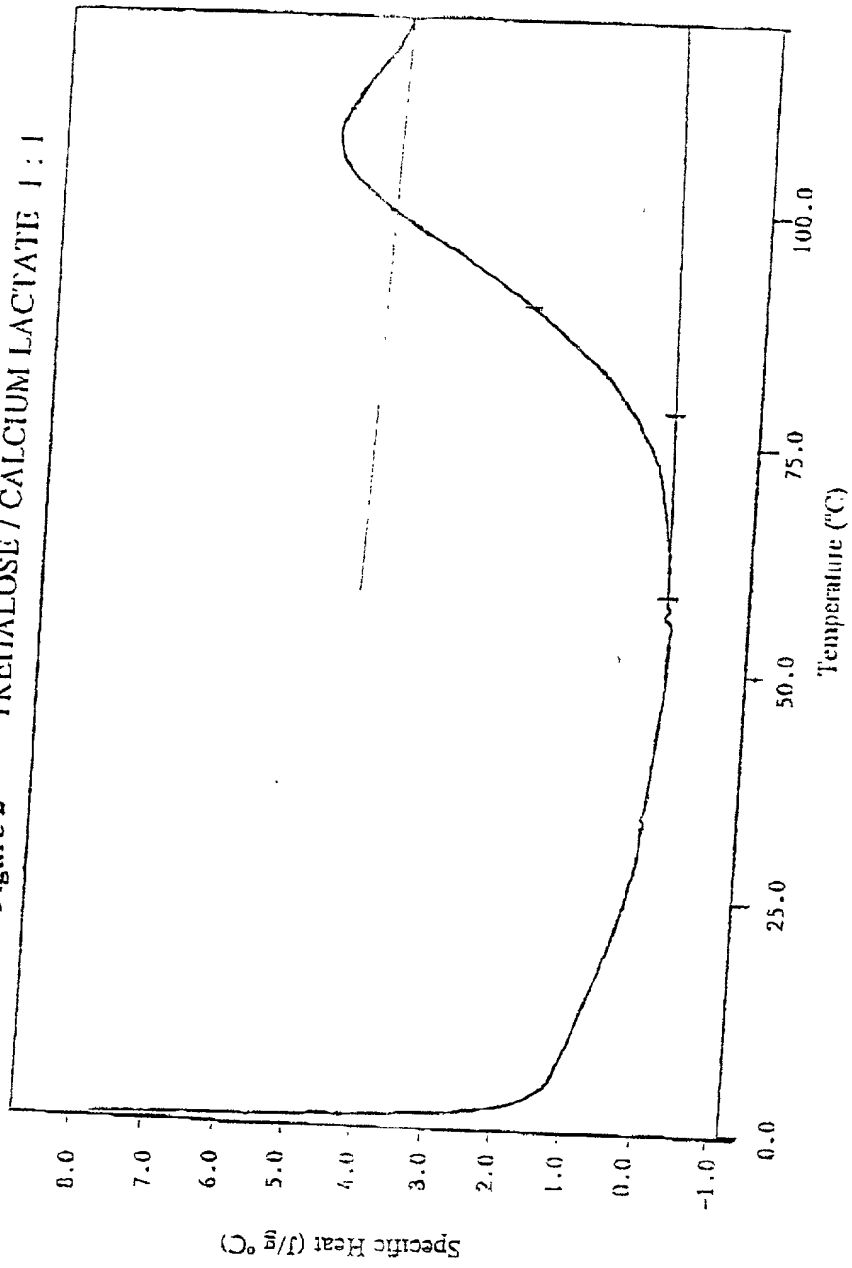

TREHALOSE / CALCIUM LACTATE 1 : 1
Specific Heat (J/g °C)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
-1.0
0.0
25.0
50.0
75.0
100.0
Temperature (°C)

Figure 2

Figure 3

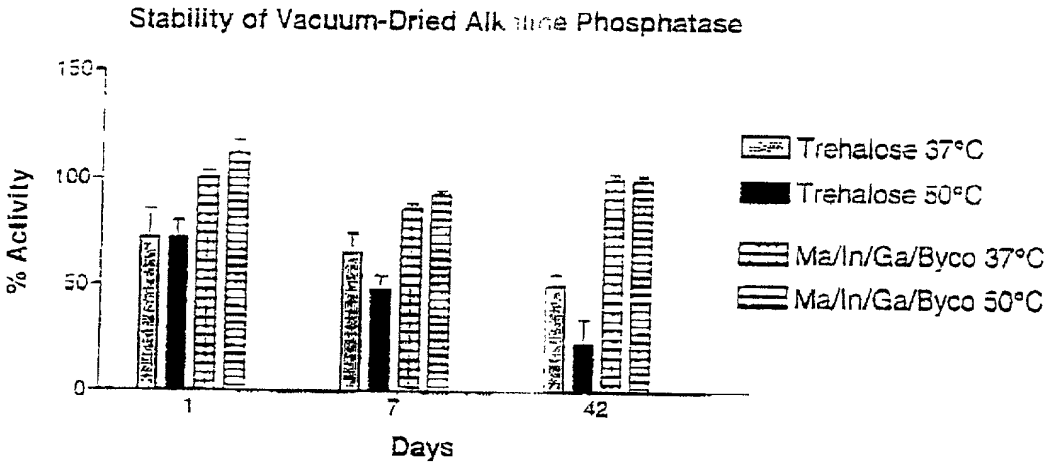

Stability of Vacuum-Dried Alkaline Phosphatase
% Activity
150
100
50
0
1
7
42
Days
Trehalose 37°C
Trehalose 50°C
Ma/In/Ga/Byco 37°C
Ma/In/Ga/Byco 50°C

Figure 4

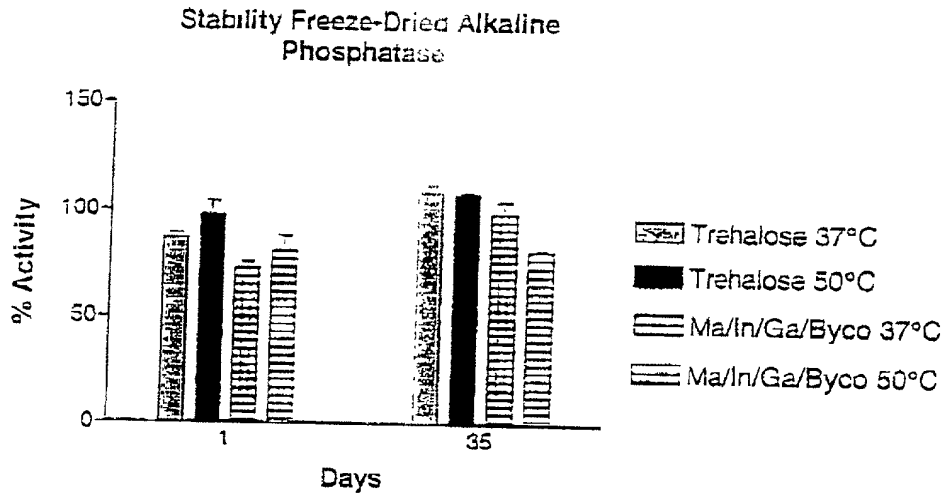

Stability Freeze-Dried Alkaline Phosphatase
% Activity
150
100
50
0
1
35
Days
Trehalose 37°C
Trehalose 50°C
Ma/In/Ga/Byco 37°C
Ma/In/Ga/Byco 50°C

Figure 5

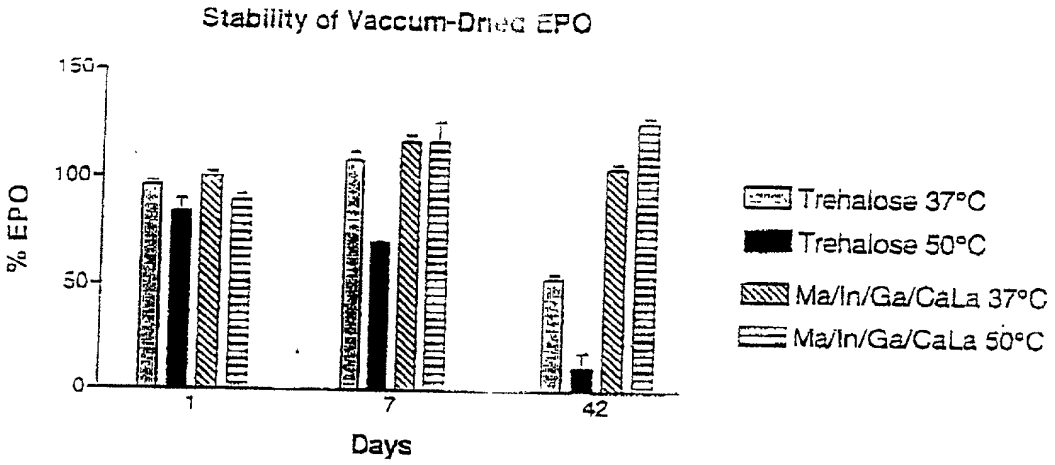
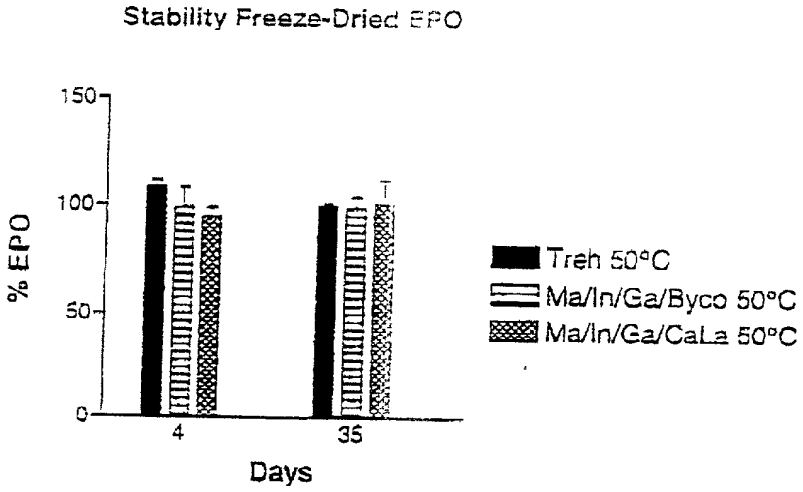

Stability of Vaccum-Dried EPO
150
100
50
0
% EPO
1
7
42
Days
Trehalose 37°C
Trehalose 50°C
Ma/In/Ga/CaLa 37°C
Ma/In/Ga/CaLa 50°C

Figure 6


Stability Freeze-Dried EPO
150
100
50
0
% EPO
4
35
Days
Treh 50°C
Ma/In/Ga/Byco 50°C
Ma/In/Ga/CaLa 50°C

Figure

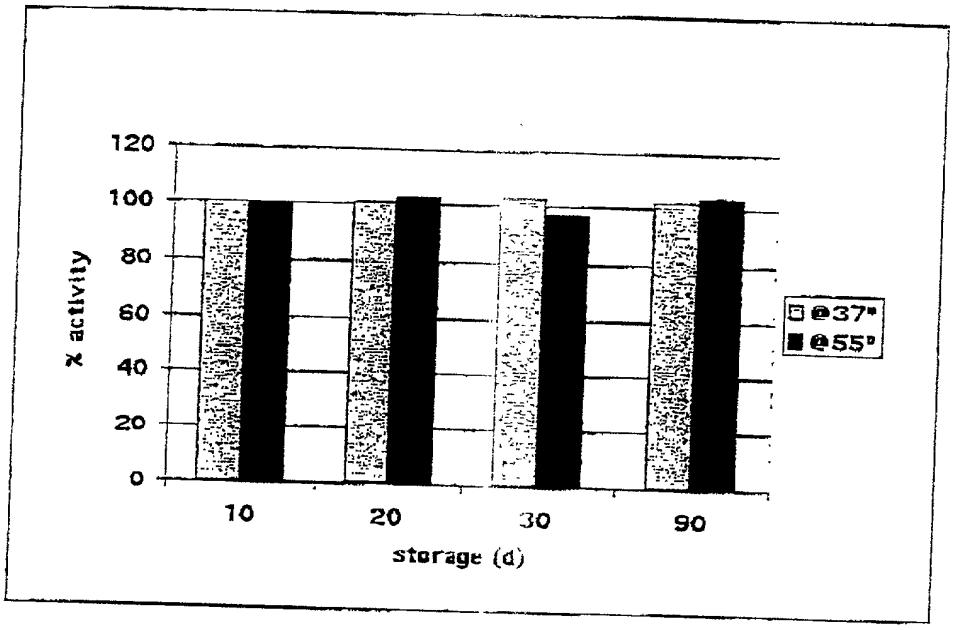
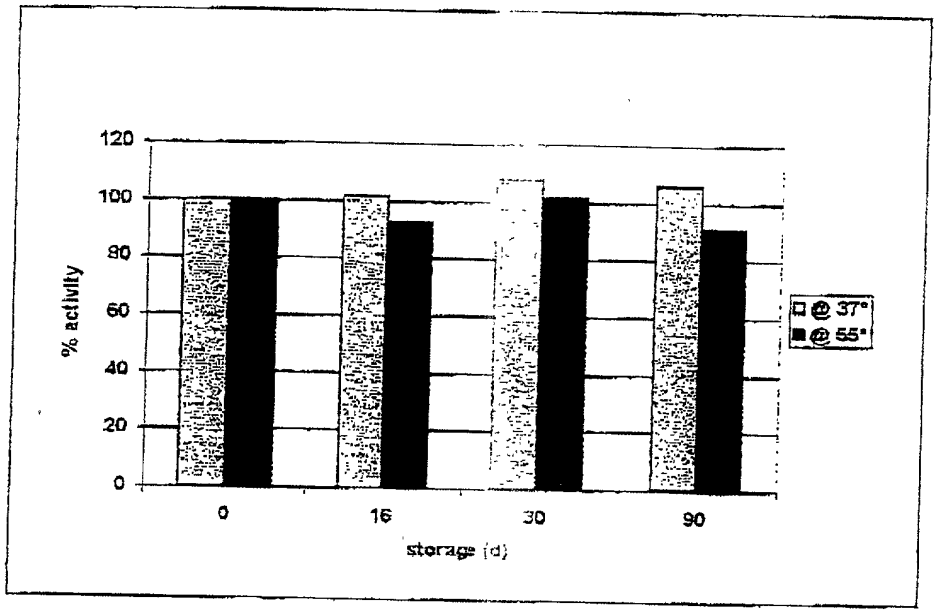


Figure 8



R&G 395670S

DECLARATION AND POWER OF ATTORNEY U.S.A.

10 Rec'd PCT/PTO 13 NOV

ATTORNEYS' DOCKET NO. 09/623495

P65952US0

ALL PATENTS, INCLUDING DESIGN
FOR APPLICATION BASED ON PCT; PARIS CONVENTION;
NON PRIORITY; OR PROVISIONAL APPLICATIONS

101 102

As a below named inventor, I declare that my residence, post office address and citizenship are stated below next to my name, the information given herein is true, that I believe that I am the original, first and sole inventor (if only one name is listed at 201 below), or an original, first and joint inventor (if plural inventors are named below at 201-203, or on additional sheets attached hereto) of the subject matter which is claimed and for which patent is sought on the invention entitled:

AMORPHOUS GLASSES FOR STABILISING SENSITIVE PRODUCTS

which is described and claimed in:

[ ] the attached specification

[X] PCT International Application No. PCT/GB99/00820 filed 17 March 1999

[X] the specification in application Serial No. 09/623,495 filed 18 September 2000

(if applicable) and

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

103

Prior Foreign Application(s)			Priority Claimed	
9805699.7 (Number)	GREAT BRITAIN (Country)	18 March 1998 (Day/Month/Year Filed)	[X] Yes	[ ] No
9820689.9 (Number)	GREAT BRITAIN (Country)	23 September 1998 (Day/Month/Year Filed)	[X] Yes	[ ] No
(Number)	(Country)	(Day/Month/Year Filed)	[ ] Yes	[ ] No

104

I hereby claim the benefit under Title 35, United States Code,§119(e) of any United States provisional application(s) listed below:

Application No. _____ Filing Date _____ Application No _____ Filing Date _____

105

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No) (Filing Date) (Status: patented, pending, abandoned)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys (Registration No.) to prosecute this application, receive and act on instructions from my agent, and transact all business in the Patent and Trademark Office connected therewith. HARVEY B. JACOBSON, JR. (20,851); D. DOUGLAS PRICE (24,514); JOHN CLARKE HOLMAN (22,769); MARVIN R. STERN (20,640); ALLEN S. MELSER (27,215); MICHAEL R. SLOBASKY (26,421); JONATHAN L. SCHERER (29,851); IRWIN M. AISENBERG (19,007), WILLIAM E. PLAYER (31,409); YOON S. HAM (45,307) and NATHANIEL A. HUMPHRIES (22,772)

SEND CORRESPONDENCE TO: CUSTOMER NO. 00136 or JACOBSON, PRICE, HOLMAN & STERN PROFESSIONAL LIMITED LIABILITY COMPANY 400 SEVENTH STREET, N.W. WASHINGTON, D.C. 20004	DIRECT TELEPHONE CALLS TO: (please use Attorney's Docket No.) (202) 638-6666 JACOBSON, PRICE, HOLMAN & STERN PROFESSIONAL LIMITED LIABILITY COMPANY

*Inventor(s) name must include at least one unabbreviated first or middle name.

201 FULL NAME * OF INVENTOR	FAMILY NAME ROSER	GIVEN NAME Bruce		MIDDLE NAME Joseph	
RESIDENCE & CITIZENSHIP	CITY Cambridge	STATE OR FOREIGN COUNTRY UNITED KINGDOM GBX		COUNTRY OF CITIZENSHIP UNITED KINGDOM	
POST OFFICE ADDRESS	POST OFFICE ADDRESS 4 Archway Court, Barton Road	CITY Cambridge	STATE OR COUNTRY UNITED KINGDOM	ZIP CODE CB3 9LW	
202 FULL NAME * OF INVENTOR	FAMILY NAME DE CASTRO	GIVEN NAME Arcadio		MIDDLE NAME Garcia	
RESIDENCE & CITIZENSHIP	CITY Cambridge	STATE OR FOREIGN COUNTRY UNITED KINGDOM GBK		COUNTRY OF CITIZENSHIP SPAIN	
POST OFFICE ADDRESS	POST OFFICE ADDRESS 86, Wulfstan Way	CITY Cambridge	STATE OR COUNTRY UNITED KINGDOM	ZIP CODE CB1 4OH	
203 FULL NAME * OF INVENTOR	FAMILY NAME	GIVEN NAME		MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE OR FOREIGN COUNTRY		COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE OR COUNTRY	ZIP CODE	

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

SIGNATURE OF INVENTOR 201*	SIGNATURE OF INVENTOR 202*	SIGNATURE OF INVENTOR 203*
DATE 26 Oct 2000	DATE 26 OCTOBER 2000	DATE

[ ] Additional inventors are named on separately numbered sheets attached hereto.